COMMUNICATION

Effect of Fruit Juices on the Oral Bioavailability of Fexofenadine in Rats

AMRITA V. KAMATH, MING YAO, YUEPING ZHANG, SAEHO CHONG

Department of Metabolism and Pharmacokinetics, Bristol-Myers-Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543

Received 17 June 2004; revised 11 August 2004; accepted 31 August 2004

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20231

ABSTRACT: Fexofenadine has been identified as a substrate for both the efflux transporter, P-glycoprotein (P-gp), as well as the influx transporter, organic anion transporting polypeptide (OATP). Clinical studies in humans showed that fruit juices reduced the oral bioavailability of fexofenadine by preferentially inhibiting OATP over P-gp. The objective of this study was to investigate the effects of fruit juices on the oral absorption of fexofenadine in rats to establish a preclinical fruit juice–drug interaction model. In rats, fexofenadine was excreted unchanged in the urine, bile, and gastrointestinal tract, indicating minimal metabolism, making it an ideal probe to study transport processes. Coadministration of fexofenadine with ketoconazole, a P-gp inhibitor, increased the oral exposure of fexofenadine by 187%. In contrast, coadministration of fexofenadine with orange juice or apple juice to rats decreased the oral exposure of fexofenadine by 31 and 22%, respectively. Increasing the quantity of orange or apple juice administered further decreased the oral exposure of fexofenadine, by 40 and 28%, respectively. This reduction in fexofenadine bioavailability was moderate compared to that seen in humans. These findings suggest that in rats fruit juices may also preferentially inhibit OATP rather than P-gp–mediated transport in fexofenadine oral absorption, albeit to a lesser extent than observed in humans. This fruit juice–drug interaction rat model may be useful in prediction of potential food–drug interactions in humans for drug candidates.
J Pharm Sci 94:233–239, 2005

Keywords: food interactions; oral absorption; transporters; organic anion-transporting polypeptide; p-glycoprotein; bioavailability

INTRODUCTION

It is becoming increasingly evident that intestinal transporters play an important role in the oral absorption of compounds, with both influx and efflux transporters influencing drug absorption processes.[1,2] Oral absorption of compounds can be limited by efflux transporters located in the intestine such as P-glycoprotein (P-gp) or the multidrug resistance-associated protein 2 (MRP2),[3,4] while influx transporters such as the organic anion transporting polypeptide (OATP) and peptide transporters (e.g., PEPT1), can aid intestinal drug absorption.[5–7] Additionally, there appears to be an overlap in the substrate specificity between the efflux transporter P-gp, and the influx transporter OATP,[5] which could lead to opposing influences on the net absorption of a shared substrate.

Fruit juices such as grapefruit juice have been shown to increase oral bioavailability in humans

Correspondence to: Amrita V. Kamath (Telephone: 609-252-5303; Fax: 609-252-6802; E-mail: amrita.kamath@bms.com)

Journal of Pharmaceutical Sciences, Vol. 94, 233–239 (2005)

by causing mechanism-based inhibition of intestinal CYP3A4 and possibly also by inhibiting P-gp–mediated intestinal efflux.[8–10] This food–drug interaction is still not completely understood. The antihistaminic compound, fexofenadine, was identified as a substrate for both P-gp and OATP.[11,12] In humans, only 5% of the oral dose of fexofenadine is metabolized, making it an ideal probe to study transport mechanisms *in vivo*.[13] Recent studies in humans using fexofenadine as a probe, showed that upon oral coadministration with fruit juices such as grapefruit, orange, and apple juice, the oral bioavailability of fexofenadine was significantly reduced.[14] If fruit juices inhibited, only P-gp, then bioavailability of fexofenadine, should have increased rather than decreased. Because fexofenadine is also a substrate for OATP,[11] the hypothesis put forth was that the decrease in fexofenadine oral bioavailability by fruit juices is a result of greater inhibition of OATP over P-gp.[14] *In vitro* studies in cells transfected with human and rat OATP transporters showed that the fruit juices (grapefruit, orange, and apple) produced relatively more potent inhibition of the OATP transporters than of P-gp.[14] However, the human OATP-A studied in this report is not expressed in human small intestine, and other OATPs may be responsible for the transport of fexofenadine in human intestine. Recent *in vitro* studies by Nozawa et al.[12] showed that fexofenadine is transported by human OATP-B, which is expressed in the human intestine,[15] although the effect of fruit juice on this transporter has not yet been evaluated.

The *in vivo* and *in vitro* studies carried out thus far suggest preferential inhibition of OATP uptake by fruit juices, making it a useful tool to study OATP transport *in vivo*. Having an animal model that replicates the findings in humans for OATP transport would be very useful in studying the role of OATP in drug absorption, and hence improve the clinical predictability based on the existing preclinical models. Therefore, the objective of the present study was to examine the effect of fruit juice on fexofenadine in rats to establish a preclinical fruit juice–drug interaction model.

MATERIALS AND METHODS

Chemicals

Fexofenadine was prepared at the Bristol-Myers-Squibb Pharmaceutical Research Institute (Princeton, NJ). Orange juice (Tropicana pure premium) and apple juice (Motts) were purchased from Wawa (Hamilton, NJ). All other chemicals used were reagent grade or better.

Animal Studies

Adult male Sprague–Dawley rats were obtained from Charles River Lab. (Wilmington, MA). All procedures were approved by the Bristol-Myers-Squibb Institutional Animal Care and Use Committee.

Routes of Excretion and Metabolism in Bile Duct-Cannulated Rats

Male Sprague–Dawley rats were surgically prepared with indwelling bile duct, duodenal, and jugular vein cannulae 2 days prior to drug administration. Rats were fasted overnight and for the duration of the study. Water was provided *ad libitum* throughout the study. During the study, control bile was infused into the duodenum at approximately 1 mL/h to avoid depletion of bile salts. Fexofenadine was administered as a 10-min intravenous infusion to two rats at a dose of 11.8 mg/kg. The formulation used was 10% ethanol in water. Urine and bile were quantitatively collected for a 9-h period. At the end of the 9-h experiment, rats were sacrificed and the gastrointestinal tract (GIT) and feces were collected. GIT, including luminal contents, and feces were homogenized with 9 volumes of water per 1 volume of GIT/feces. Samples were analyzed for fexofenadine by LC/MS/MS.

Effect of Fruit Juices on the Oral Exposure of Fexofenadine in Rats

Rats were surgically prepared with an indwelling jugular vein cannula. Animals were fasted overnight prior to dosing and fed approximately 6 h postdose. Water was provided *ad libitum* throughout the study. The pharmacokinetics of fexofenadine were investigated following a single dose of approximately 10 mg/kg fexofenadine (which corresponds to the clinical dose of 120 mg in humans) given orally by gavage with orange juice, apple juice, water, or 10 mg/kg of ketoconazole ($n = 3$ rats per group, except the orange juice group where $n = 6$ rats). The dose volume given was 10 mL/kg, and dosing solution consisted of 80%

fruit juice (or water): 20% ethanol. Blood samples were collected at 15, 30, 75 min, 1, 2, 4, 6, 8, and 10 h after oral dosing. In a second study ($n = 3$ rats per group), the amount of fruit juice administered was increased from 8 mL/kg to 28 mL/kg, and given in divided doses over 1 h. These groups were designated as "extra fruit juice" groups. The volume and schedule of fruit juice administration was as follows: 10 mL/kg of juice, followed by 10 mg/kg of fexofenadine after 30 min at 10 mL/kg in 80% juice:20% ethanol, followed by 10 mL/kg of juice after another 30 min. Blood samples were collected at 15, 30, and 75 min, 1, 2, 4, 6, 8, and 10 h postfexofenadine administration. In both studies approximately 0.25 mL of blood was collected from the jugular vein catheter in heparinized tubes and plasma was obtained by centrifugation. Samples were analyzed for fexofenadine by LC/MS/MS analysis.

Sample Analysis

Fexofenadine concentrations in plasma, bile, urine, and GIT samples from rat studies were determined using a LC/MS/MS method. Samples (100 μL) were treated with 50 μL of ammonium chloride buffer, pH 8.8, 300 μL of acetonitrile containing 500 ng/mL of oleandrin as an internal standard (IS), and 150 μL of methylene chloride. After centrifugation, the supernatant was transferred to a glass tube and dried under nitrogen. The residue was reconstituted with 75 μL of the mobile phase, and a 20-μL portion was injected onto the LC/MS. The HPLC column (50×2.0 mm) was a 3-micron YMC ODS AQ analytical column from Waters Corporation (Milford, MA). The mobile phase consisted of 50:50 (v/v) acetonitrile and 5 mM ammonium formate, pH 3.4. Chromatography was performed isocratically at a flow rate of 0.2 mL/min at room temperature. The HPLC was interfaced with a Sciex API3000 LC/MS/MS system with an atmospheric pressure ionization (API) and electrospray inlet in the positive ion-multiple reaction monitoring mode. The ions monitored were precursor $\rightarrow$ product ions of m/z $502.59 \rightarrow 466.26$ for fexofenadine and m/z $577.6 \rightarrow 433.3$ for oleandrin (IS). The API3000 instrument parameters were (arbitrary units): CAD:4, DP:38, FP:180, EP:-10, CE:38, CXP:25, IS:5000, and DF:-400. Additional settings optimized using the heated nebulizer interface were as follows (arbitrary units): NEB:11 and CUR:11. The temperature was set at 400°C.

Data Analysis

The pharmacokinetic parameters for fexofenadine were derived by noncompartmental methods[16] using the KINETICA™ software program. The C_{max} and T_{max} values were recorded directly from experimental observations. The AUC_{0n} values (area under the concentration versus time curve from 0 to time of the last measurable concentration) were calculated using a combination of linear and log trapezoidal rules. Statistical analysis were performed where appropriate using a two-tailed unpaired t-test with significance set at $p < 0.05$.

RESULTS

Routes of Excretion and Metabolism in Bile Duct Cannulated Rats

The recovery of fexofenadine in bile, urine, and GIT of bile duct cannulated rats is summarized in Table 1. After an intravenous dose of 11.8 mg/kg in bile duct-cannulated rats, almost all of the dose was recovered in the urine, bile, and gastrointestinal tract as an unchanged drug. The dose was chosen to be equivalent to the 120-mg human dose used in the clinical study by Dresser et al.[14] These results demonstrate that fexofenadine is excreted unchanged in rats. Metabolism does not play a major role in the elimination of fexofenadine, making it an ideal probe to study transport processes in the rat.

Effect of Fruit Juices on the Oral Exposure of Fexofenadine in Rats

Pharmacokinetic parameters in the rat after oral administration of fexofenadine in the presence or absence of fruit juice are summarized in Table 2 and the plasma concentration–time profiles are presented in Figure 1. On coadministration of an

Table 1. Recovery of Fxofenadine after an Intravenous Dose to Bile Duct Annulated Rats

Parameter	Rat 1	Rat 2
Dose (mg/kg)	11.8	11.8
% dose in urine (0–9 h)	15.7	19.2
% dose in bile (0–9 h)	64.0	75.5
% dose in GIT (9 h)	5.6	5.9
Total recovery as % of dose	85.3	100.6

Table 2. Pharmacokinetic Parameters of Fexofenadine in Rats (Mean ± SD)

Parameter	Water Control ($n = 3$)	Ketoconazole ($n = 3$)	Orange Juice ($n = 6$)	Apple Juice ($n = 3$)	Extra Orange Juice[a] ($n = 3$)	Extra Apple Juice[a] ($n = 3$)
Dose (mg/kg)	10	10	10	10	10	10
C_{max} (nM)	49 ± 2	159 ± 66^b	27 ± 5^b	37 ± 16	19 ± 8^b	21 ± 7^b
T_{max} (h)	0.3 ± 0	0.5 ± 0.3	0.6 ± 0.1	0.7 ± 0.4	2.2 ± 1.8	0.5 ± 0.3
AUC(0–10 h) (nM · h)	148 ± 12	423 ± 91^b	102 ± 26^b	115 ± 38	89 ± 23^b	106 ± 42
% Change in AUC from control	—	187%	−31%	−22%	−40%	−28%

[a]Extra fruit juice: amount of fruit juice administered was increased from 8 mL/kg as a single dose to 28 mL/kg in divided doses over 1 h as described in the 'Materials and Methods' section.
[b]$p < 0.05$.

oral dose of fexofenadine with 10 mg/kg of ketoconazole (a P-gp inhibitor) the exposure of fexofenadine (C_{max} and AUC) increased significantly. This showed that ketoconazole inhibited the P-gp–mediated efflux of fexofenadine during absorption, and/or excretion via the biliary route. In contrast, administration of orange juice or apple juice to rats caused a decrease in the oral exposure of fexofenadine. The AUC(0–10 h) of fexofenadine decreased by 31 and 22% upon coadministration with orange juice or apple juice, respectively, suggesting that an influx transporter like OATP may be inhibited. After increasing the amounts of orange juice or apple juice given with a single dose of fexofenadine, the oral exposure of fexofenadine was further decreased. The AUC(0–10 h) of fexofenadine decreased by 40 and 28% with the extra amounts of orange juice or apple juice, respectively.

DISCUSSION

The oral absorption of a compound is dependent on several factors including the characteristics of the compound (e.g., solubility and lipophilicity), as well as its interaction with the intestinal epithelium.[2] Efflux proteins located on the intestinal epithelium like P-gp or MRP2 act to limit the absorption of compounds that are substrates thereby decreasing their bioavailability.[1,3,4] Influx transporters like OATP or PEPT1, on the other hand, act to facilitate the absorption of compounds that are substrates of those transporters.[3,5] Some OATP substrates also appear to be substrates of P-gp and MRP2.[5] Overlapping substrate specificities between influx and efflux transporters could affect the net absorption of the compound. Additionally, intestinal metabolism by drug metabolizing enzymes expressed in the gut wall contribute to lowering the bioavailability of an orally administered drug.[2]

Grapefruit juice has been shown to increase the oral bioavailability of several therapeutic compounds like felodipine, nifedipine, saquinavir, and sildenafil.[8–10,17,18] This increase has been shown to be due to mechanism-based inhibition of intestinal CYP3A4 drug metabolism by grapefruit juice.[9,18] In addition to causing inhibition of CYP3A4 metabolism, grapefruit juice has also been implicated in inhibiting intestinal P-gp activity of certain compounds like cyclosporine, thereby increasing its oral bioavailability.[5,9]

In vitro and *in vivo* studies have shown that several fruit juices like grapefruit, orange, and apple juice produced more potent inhibition of OATP transporters over P-gp.[14,19] *In vitro* studies conducted by Dresser et al.[14] showed that grapefruit, orange, and apple juice at 5% of normal strength did not alter P-gp activity, but significantly reduced human OATP and rat Oatp activity. Constituents of grapefruit juice like 6′, 7′-dihydroxybergamottin and naringin at concentrations of 33 and 3000 μM, respectively, reduced *in vitro* P-gp activity by 50%, while at a lower concentration of 5 μM, they caused 50% inhibition of rat Oatp3 activity.[14] Dresser et al.[14] also showed that 6′,7′-dihydroxybergamottin inhibited rat Oatp1, expressed in the proximal colon, with an IC_{50} of 0.28 μM, while IC_{50} for P-gp inhibition was much higher at 33 μM. These results strongly suggest that several furanocoumarins and bioflavanoids present in fruit juices are more potent inhibitors of OATP transporters than of P-gp. However, this report[14] only showed fruit juice effects on human OATP-A, which is not expressed in human intestine. Further studies are needed to demonstrate effect of fruit juices on human OATP transporters such as OATP-B, which is expressed in human intestine.[15]

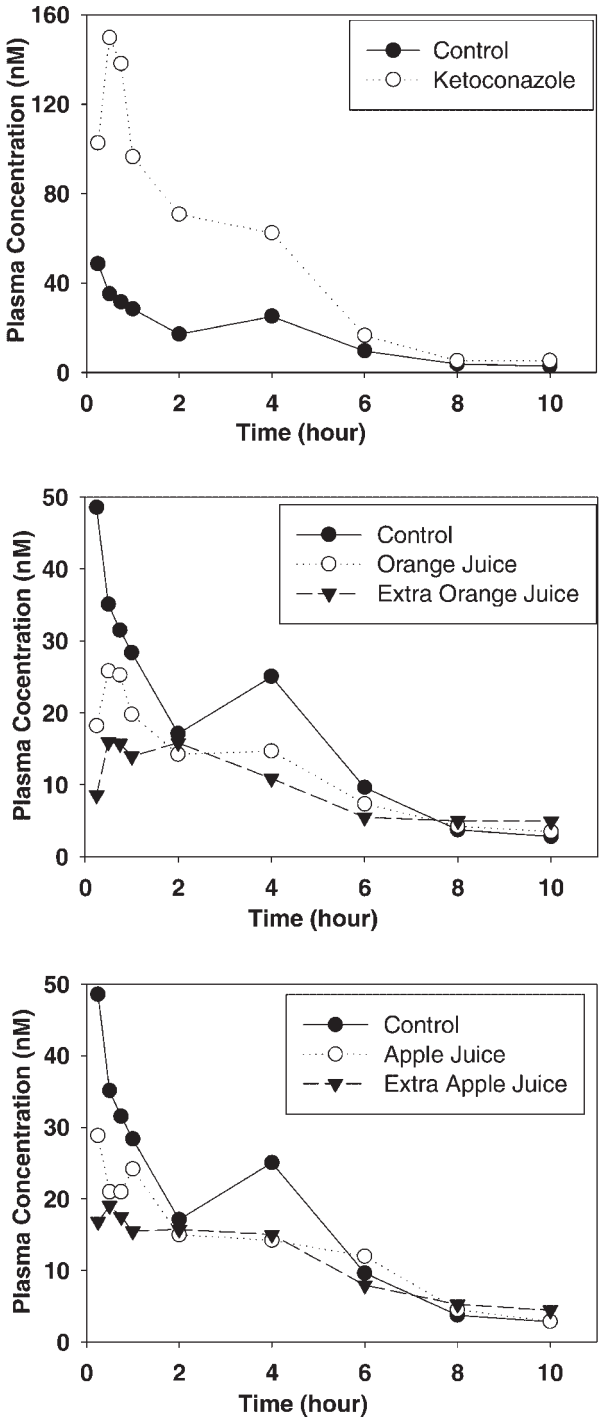

Figure 1. Plasma concentration versus time profiles in rats after a single oral dose of 10 mg/kg fexofenadine in the absence (control) and presence of 10 mg/kg of ketoconazole, or single or multiple doses of orange juice and apple juice (mean values). In the "Extra fruit juice" groups, the amount of fruit juice administered was increased from 8 mL/kg as a single dose to 28 mL/kg in divided doses over 1 h as described in the Materials and Methods section.

In previous studies, fexofenadine has been shown to be a substrate of the efflux transporter, P-gp,[11] as well as the influx transporter, OATP.[11,12] Both human OATP-A and rat Oatp1 and Oatp2 were found to mediate fexofenadine cellular uptake.[11] Fexofenadine was also found to be a substrate of the rat Oatp3 transporter, which is highly expressed on the brush border membrane of enterocytes.[19,20] Recent *in vitro* studies by Nozawa et al.[12] showed that fexofenadine is also transported by human OATP-B, which is expressed in the human intestine.[15] In the present study, coadministration of fexofenadine with a P-gp inhibitor, ketoconazole, significantly increased the systemic exposure of fexofenadine in rats. This is similar to the results obtained in humans where coadministration of 120 mg of fexofenadine with 400 mg of ketoconazole led to a 164% increase in fexofenadine exposure.[13] In contrast, coadministration of orange juice or apple juice with fexofenadine caused a decrease in the oral exposure (AUC and C_{max}) of fexofenadine in rats, suggesting preferential inhibition of OATP over P-gp. This observation is also consistent with results in humans. However, the extent of decrease in the oral bioavailability in rats was not as great as that observed in humans. Increasing the amount of coadministered orange or apple juice caused a further decrease in the systemic exposure of fexofenadine, which was still not as great as observed in humans. The dose of 10 mg/kg used in this study corresponds to the 120 mg human dose used in the clinical study by Dresser et al.[14] The large volumes of fruit juice could have caused alterations in the intestinal local environment in rats. However, a large volume of fruit juice (total volume of 1.2 L) was also administered in the clinical study.[14] Presumably, changes in the intestinal local environments due to fruit juices, affecting fexofenadine absorption, would be similar in rats and humans. However, Dresser et al.[14] showed that even when smaller volumes of 300 mL were administered to humans, decreases in plasma concentrations of fexofenadine were observed. These results suggest that the volume of fruit juices used may not have contributed significantly to a moderate decrease in fexofenadine oral exposure in rats compared to humans, by causing changes in its physicochemical properties. Further studies examining the bioavailability of fexonadine with individual constituents of fruit juices will be useful in gaining a better understanding of the transport processes involved in its absorption.

The noncrossover design employed in these rodent studies may be one of the reasons for the moderate reduction in fexofenadine oral bioavailability compared to that observed in humans. A second more likely reason may be the differences in transporter activities between rats and humans. The affinity and capacity of fexofenadine for the intestinal OATP/Oatps could be different in the rat (e.g., rat Oatp3) and human (e.g., OATP-B). The K_m value of fexofenadine for rat Oatp-3 was 36 μM;[19] however, the affinity of fexofenadine for human OATP-B has not yet been determined. A third possibility is the effect of pH on fexofenadine absorption. Fexofenadine exists as a zwitterion in aqueous media at physiological pH.[13] The ingestion of orange juice and apple juice, which are at an acidic pH, could lower the pH in the intestinal lumen and may affect the function of uptake transporters of fexofenadine. Recent reports[12,15] showed that OATP-B, which is expressed in human intestine, exhibits pH-sensitive transport for various organic anions. *In vitro* cellular studies[12] showed that while fexofenadine was transported by OATP-B at both pH 7.4 and pH 5.0, higher activity was observed at the acidic pH. The pH values in the upper small intestine has been reported[21] to be higher in rats (pH 6.5) compared to that in humans (pH 5.4). These differences in intestinal pH values in rats and human may have contributed to the differences seen between rats and humans.

In summary, these findings suggest that fruit juices may also be preferentially inhibiting OATP rather than P-gp–mediated transport of fexofenadine in rats, albeit to a lesser extent than observed in humans. This decrease in exposure could result in reduced effectiveness of fexofenadine in patients. This fruit juice–drug interaction rat model may be useful in prediction of potential food–drug interactions in humans for drug candidates.

ACKNOWLEDGMENTS

The assistance of the Technical Support Unit in animal maintenance, dosing, and sample collection, is greatly appreciated.

ABBREVIATIONS

API: atmospheric pressure ionization
AUC0n: area under the concentration *vs*. time curve from 0 to time of the last measurable concentration
Cmax: maximum concentration
GIT: gastrointestinal tract
IS: internal standard
MRP2: multidrug resistance-associated protein 2
OATP: organic anion transporting polypeptide
PEPT1: peptide transporter 1
P-gp: p-glycoprotein
Tmax: time to reach Cmax

REFERENCES

1. Kunta JR, Sinko PJ. 2004. Intestinal drug transporters: In vivo function and clinical importance. Curr Drug Metab 5:109–124.
2. Pang KS. 2003. Modeling of intestinal drug absorption: Roles of transporters and metabolic enzymes (for the Gillette Review Series). Drug Metab Dispos 31:1507–1519.
3. Chan LM, Lowes S, Hirst BH. 2004. The ABCs of drug transport in intestine and liver: Efflux proteins limiting drug absorption and bioavailability. Eur J Pharm Sci 21:25–51.
4. Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, Wilkinson GR. 1998. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest 101:289–294.
5. Kim RB. 2003. Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. Eur J Clin Invest 33:1–5.
6. Terada T, Inui K. 2004. Peptide transporters: Structure, function, regulation and application for drug delivery. Curr Drug Metab 5:85–94.
7. Tirona RG, Kim RB. 2002. Pharmacogenomics of organic anion-transporting polypeptides (OATP). Adv Drug Deliv Rev 54:1343–1352.
8. Bailey DG, Malcolm J, Arnold O, Spence JD. 1998. Grapefruit juice–drug interactions. Br J Clin Pharmacol 46:101–110.
9. Dresser GK, Bailey DG. 2003. The effects of fruit juices on drug disposition: A new model for drug interactions. Eur J Clin Invest 33:10–16.
10. Fuhr U. 1998. Drug interactions with grapefruit juice. Extent, probable mechanism and clinical relevance. Drug Saf 18:251–272.
11. Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, Kim RB. 1999. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. Drug Metab Dispos 27: 866–871.
12. Nozawa T, Imai K, Nezu J, Tsuji A, Tamai I. 2004. Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. J Pharmacol Exp Ther 308:438–445.

13. ALLEGRA (fexofenadine hydrochloride). 2003. Physician's Desk Reference.
14. Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, Kim RB. 2002. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther 71:11–20.
15. Kobayashi D, Nozawa T, Imai K, Nezu J, Tsuji A, Tamai I. 2003. Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. J Pharmacol Exp Ther 306:703–708.
16. Gibaldi M, Perrier D. 1982. Pharmacokinetics. New York: Marcel Dekker.
17. Bailey DG, Spencer JD, Munoz C, Arnold JM. 1991. Interaction of citrus juices with felodipine and nifedipine. Lancet 337:268–269.
18. Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, Brown MB, Guo W, Watkins PB. 1997. Grapefruit Juice increases felodipine oral availability in humans by decreasing intestinal CYP3A4 protein expression. J Clin Invest 99:2545–2553.
19. Dresser GK, Schwarz UI, Leake B, Choo EF, Wilkinson GR, Bailey DG, Kim RB. 2001. Citrus Juices are potent inhibitors of intestinal OATP but not p-glycoprotein. Clin Pharmacol Ther 69:P23 (abstract).
20. Walters HC, Craddock AL, Fusegawa H, Willingham MC, Dawson PA. 2000. Expression, transport properties, and chromosomal location of organic anion transporter subtype 3. Am J Physiol 279:G1188–G1200.
21. Davies B, Morris T. 1993. Physiological parameters in laboratory animals and humans. Pharm Res 10:1093–1095.